all-atom and coarse-grained molecular dynamics simulations, we have explored the membrane-binding behavior of several lipopeptide families, including the Shai peptides, Lfb6, and fengycins. These simulations, validated via careful comparison with experiment, help to reveal the atomic-level mechanism for their function, yielding insights needed for rational design of better antibacterial and antifungal agents.

56-Subg
Fatty Acids and Lysolipids Perturb Lipid Membranes: Implications for Drug Delivery
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Fatty acids and lysolipids incorporate into lipid membranes and may exert an effect on their permeability, morphology, and stability, leading, e.g., to a reduction in the permeability barrier. The origin of this phenomenon may be related to changes in the curvature stress of the membrane caused by the effective non-cylindrical geometry of fatty acids and lysolipids as compared to cylindrical phospholipids. It has been proposed that the same effects may carry over to apply for the permeability barrier of cell membranes, in which case the effect could possibly be exploited to enhance intracellular drug uptake. However, fatty acids and lysolipids are in themselves cytotoxic in micromolar concentrations and can induce cell lysis and apoptosis. Experiments with living cells have shown that fatty acids and lysolipids at concentrations below their cytotoxicity limit cannot render cell membranes more permeable by perturbing the lipid-bilayer component of the membrane. This implies that development of liposomal drug-delivery systems, e.g., those using endogenous phospholipase activity as a trigger to unload drugs, are faced with the problem of overcoming the barrier for transferring active drugs across the target membranes.
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57-Subg
Both Detergent Effects Upon Domain Size and Transmembrane Protein Length Effects Upon Domain Binding Suggest that Hydrophobic Mismatch can Control the Properties of Ordered Membrane Domains ("Rafts")
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Hydrophobic mismatch between co-existing domains potentially plays an important role in determining domain size and domain interaction with transmembrane proteins. We recently found that in bilayers with the potential to form co-existing Ld and Lo domains, the detergent Triton X-100 enlarges ordered domain size without perturbing ordered domain formation. This can be explained if the effect of detergent is greatest at the boundary between Lo and Ld domains, which would be the case if the main effect of detergent bound to the membranes is to alter the difference between Lo and Ld bilayer width. In other studies, we found that for a multi-transmembrane segment protein, perfringolysin O (PFO), altering the length of transmembrane sequences controls affinity of the protein for membranes domains in a fashion that is dependent upon domain bilayer width. In bilayers with co-existing Ld and Lo domains, PFO with shortened transmembrane segments preferred to partition into the membrane domains with a thin bilayer width, while PFO with lengthened transmembrane segments preferred to partition into domains forming a wider bilayer. PFO with intermediate length, wild type, transmembrane segments exhibited intermediate behavior. This relative bilayer width preference was the same whether the Lo domains were the thinner or wider membrane domains. The effect of transmembrane length upon domain localization was observed both in vesicles that have domains large enough to see by light microscopy and in vesicles with sub-microscopic domains, in which domain affinity was assayed with FRET. Thus, for both of detergent effects and protein-domain association experiments it is likely that hydrophobic mismatch is a key parameter.

58-Subg
Functional Reconstitution of Membrane Proteins by Isothermal Titration Calorimetry
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Membrane proteins make up roughly 30% of all proteins encoded by the human genome and represent about 50% of drug targets in the human body. They fulfil vital functions as receptors and signal transducers, channels and transporters, motors and anchors. Many of these functions are amenable to biochemical and biophysical investigation only after the membrane protein of interest has been extracted, purified, and reconstituted into artificial liposomes. Extraction from the host-cell membrane and chromatographic purification are usually performed with the aid of detergents. However, detergent micelles do not allow the study of vectorial functions such as solute transport or signal transduction. Therefore, numerous membrane proteins need to be reconstituted from a purified, detergent-solubilised state into liposomes in order to regain their native structures and activities.
Unfortunately, functional reconstitution has remained one of the main bottlenecks in the handling of membrane proteins. In particular, gauging the success of reconstitution experiments has thus far been limited to trial-and-error approaches. To address this problem, we have established high-sensitivity isothermal titration calorimetry (ITC) as a powerful method for monitoring the reconstitution of membrane proteins into liposomes. ITC has previously been employed for characterising liposome solubilisation and reconstitution in the absence of protein [1,2]. Recent work in our laboratory has demonstrated that ITC is also excellently suited for tracking the complex process of membrane-protein reconstitution in a non-invasive and fully automated manner. The approach is exemplified for the prokaryotic potassium ion channel KcsA, which was functionally reconstituted into stable proteoliposomes at high protein densities. Electrophysiological experiments confirmed that KcsA regained its functional activity upon ITC-guided reconstitution.
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59-Subg
Detergents for Extraction, Purification, and Reconstitution of G Protein-Coupled Membrane Receptors
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G protein-coupled membrane receptors (GPCR) are, perhaps, the most important signaling molecules for the transfer of external signals to the cell interior. The rhodopsin-like receptors are the largest class of GPCR. They are integral membrane proteins with seven transmembrane helices, including not only receptors for vision, but also receptors for neurotransmitters, chemokines, neuropeptides, cannabinoids, lysolipids, prostaglandins, histamines, and odorants, just to mention a few. A still rather limited number of rhodopsin-like GPCR have been successfully overexpressed in various expression systems, solubilized with the help of detergents, purified in quantities up to milligrams, and reconstituted into lipid bilayers for functional and structural studies. Every one of those steps requires the use of detergents. Detergents are also used for crystallization of GPCR, although this will not be subject of my presentation. All GPCR are known to be highly vulnerable to denaturation while solubilized with detergents. The successful laboratories have spent years optimizing experimental conditions to increase yield of purified GPCR. A significant fraction of this process is identifying proper detergents for work with a particular GPCR. Most of this expertise was gained by trial and error. By now, patterns in the use of detergents have emerged that are applicable to more than one of the GPCR. In my talk, I will present experience gained with rhodopsin and the recombinant, type II cannabinoid receptor at my laboratory, but will also review literature on the use of detergents for other GPCR. I hope that my attempt of summarizing practical observations on GPCR and detergent use will stimulate discussions that eventually lead to a more purposeful selection of detergents in the future.

Subgroup: Exocytosis & Endocytosis

60-Subg
Molecular Basis and Physiological Consequences of Synaptic Vesicle Pool Heterogeneity
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In this presentation I will discuss our recent studies on the molecular basis and physiological consequences of synaptic vesicle pool heterogeneity.